

9-27-2009

Stormwater Irrigation Volume III: Transport Of The Cyanotoxin Microcystin In Groundwater Beneath Stormwater Ponds: Results Of Soil Column Experiments

Martin Wanielista
University of Central Florida



Part of the [Environmental Engineering Commons](#)

Find similar works at: <https://stars.library.ucf.edu/bmptrains-research>

University of Central Florida Libraries <http://library.ucf.edu>

This Publication is brought to you for free and open access by the Stormwater Management Academy at STARS. It has been accepted for inclusion in BMP Trains Research and Publications by an authorized administrator of STARS. For more information, please contact STARS@ucf.edu.

Recommended Citation

Wanielista, Martin, "Stormwater Irrigation Volume III: Transport Of The Cyanotoxin Microcystin In Groundwater Beneath Stormwater Ponds: Results Of Soil Column Experiments" (2009). *BMP Trains Research and Publications*. 25.

<https://stars.library.ucf.edu/bmptrains-research/25>





STORMWATER IRRIGATION VOLUME III:
TRANSPORT OF THE CYANOTOXIN MICROCYSTIN IN GROUNDWATER
BENEATH STORMWATER PONDS:
RESULTS OF SOIL COLUMN EXPERIMENTS

Prepared By:
Marty Wanielista, PhD. P.E.



FDEP Project Number: S0134
September 27, 2009

ACKNOWLEDGEMENT:

This research was supported through an Urban Nonpoint Source Research Grant from the Bureau of Watershed Restoration, Florida Department of Environmental Protection.

Support from the stormwater Management Academy and students at the University of Central Florida is also appreciated.



© 2009

Stormwater Irrigation Volume III: Transport of the Cyanotoxin Microcystin in Groundwater beneath Stormwater Ponds: Results of Soil Column Experiments

Abstract

As the demand for fresh water increases in central Florida to meet both public supply and irrigation needs, stormwater is increasingly being managed as a resource to help offset possible future declines in aquifer water levels. Water quality is an important consideration when using stormwater for recharge or harvesting. A constituent of recent concern is cyanobacteria (popularly known as blue-green algae) because some of these can produce toxins (cyanotoxins) that are detrimental to animal and human health. Microcystins are the most commonly found type of cyanotoxin in Florida and have been detected in a variety of rivers, natural lakes, and stormwater ponds. Filtration of stormwater through natural or amended soil sediments and withdrawal of the filtered water through horizontal wells beneath the pond are probable technologies for mitigation of water quality concerns. The potential for transport of microcystin in soil and ground water was investigated by using laboratory soil column experiments.

Results of two soil column experiments indicate the potential for substantial removal of microcystin during saturated flow through sand. Natural stormwater spiked to yield microcystin concentration of 3.9 and 2.2 micrograms per liter was continuously applied during each experiment lasting 3 and 10 days, respectively. Samples were collected from six sampling ports at varying depths along each column in addition to the ponded water at the top of each column. Samples were quantitatively analyzed using enzyme-linked immunosorbent assay (ELISA). Concentration-based microcystin removal efficiencies up to 90 percent and mass-balance based removal efficiencies up to 70 percent were achieved. Breakthrough curves indicated relatively conservative transport of microcystin at breakthrough, suggesting sorption processes were limited, followed by substantial declines in concentration, suggesting kinetic reaction processes were important and possibly caused by microbial degradation. Microcystin losses generally increased with depth up to 2 feet and remained relatively constant at greater depths. Microbial degradation likely can be attributed mostly to the biofilm layer that formed on the sand surface, causing the large microcystin losses in the upper parts of the columns. However, breakthrough curves indicated generally lower concentrations in deeper sampling ports, suggesting additional removal capacity was afforded by the thicker filter bed. Based on the soil and water quality conditions used in the experimental setup, a sand filter bed ranging from 2 to 4 feet in thickness was found to be sufficient for effective microcystin removal.

Background

Stormwater is increasingly being managed as a resource to help offset possible future declines in aquifer water levels as the demand for fresh water increases in central Florida to meet both public supply and irrigation needs. Water quality is an important consideration when using stormwater for public-access irrigation. A constituent of recent concern is cyanobacteria (popularly known as blue-green algae), because some cyanobacteria produce toxins (cyanotoxins) that are detrimental to animal and human health (Abbott et al, 2009). Filtration of stormwater through natural or amended soil sediments and withdrawal of the filtered water through horizontal wells beneath the pond is a potential technology for mitigation of water quality concerns. However, the biogeochemical processes controlling the transport and fate of cyanotoxins in soil and ground water are not well known.

Microcystins are a commonly found type of cyanotoxin in Florida and have been detected in a variety of Florida water bodies, including rivers, natural lakes, and reservoirs (Burns et al, 2002; Abbott et al, 2009). Recent research by the University of Central Florida indicates that cyanobacteria and microcystins also are present in stormwater ponds. Of the 15 stormwater ponds sampled in Orange County, all had detectable levels of microcystin with concentrations ranging from 0.04 to 1.56 micrograms per liter (ug/L) (Wanielista et al, 2006, p. 30). Other cyanotoxins have also been reported in Florida, including cylindrospermopsin, anatoxin-a, and lyngbyatoxin (Burns et al, 2002).

Microcystin-containing genera of cyanobacteria retain the toxin within healthy cells; toxin is released when cells lyse. Once released into the water, the dissolved microcystins can potentially move with the prevailing hydraulic gradient through the bottom sediments and into adjacent aquifers. Wanielista et al (2006) conducted research whereby microcystin-containing stormwater was infiltrated through 4 feet (ft) of poorly graded, natural, sandy soil in a 2 by 2 ft chamber and reported a reduction in microcystin concentration of the percolate. Other research involving column experiments (Lahti et al, 1996) and field sampling of ground water at a bank filtration facility (Grützmacher et al, 2002b) also demonstrated the potential for microcystin attenuation or degradation in soil and ground water.

Objective

The objective of this study is document the potential for transport of the cyanobacterial hepatotoxin microcystin in soil and ground water by using laboratory soil column experiments. This report documents the experimental methods and data derived during two soil column experiments.

Experimental Methods

Two soil column experiments were performed using microcystin-LR (MC-LR) and a clean sand media. MC-LR is the most commonly occurring and one of the most toxic

microcystin congeners (Svrcek and Smith, 2004, p. 159). Natural stormwater spiked with a commercially available MC-LR standard (Abraxis, Warminster, PA) was continuously applied to two (duplicate) columns for the duration of each experiment.

Column Construction and Operation

Three identical columns were constructed of cast acrylic tubes (nominal 5-ft tall, 11.5-inch inside diameter); polytetrafluoroethylene (PTFE) or perfluoroalkoxy (PFA) fittings and tubing were used for the sampling ports (Figure 1). Acrylic and PTFE/PFA materials were chosen for their chemical inertness, thus minimizing interaction between MC-LR and column materials. Six sampling ports were installed at varying depths along each column and identified by letter (Figure 1); port inlets extend to the center of the column to minimize column sidewall effects. Photographs of a completed soil column and sampling port are shown in Figure 2.

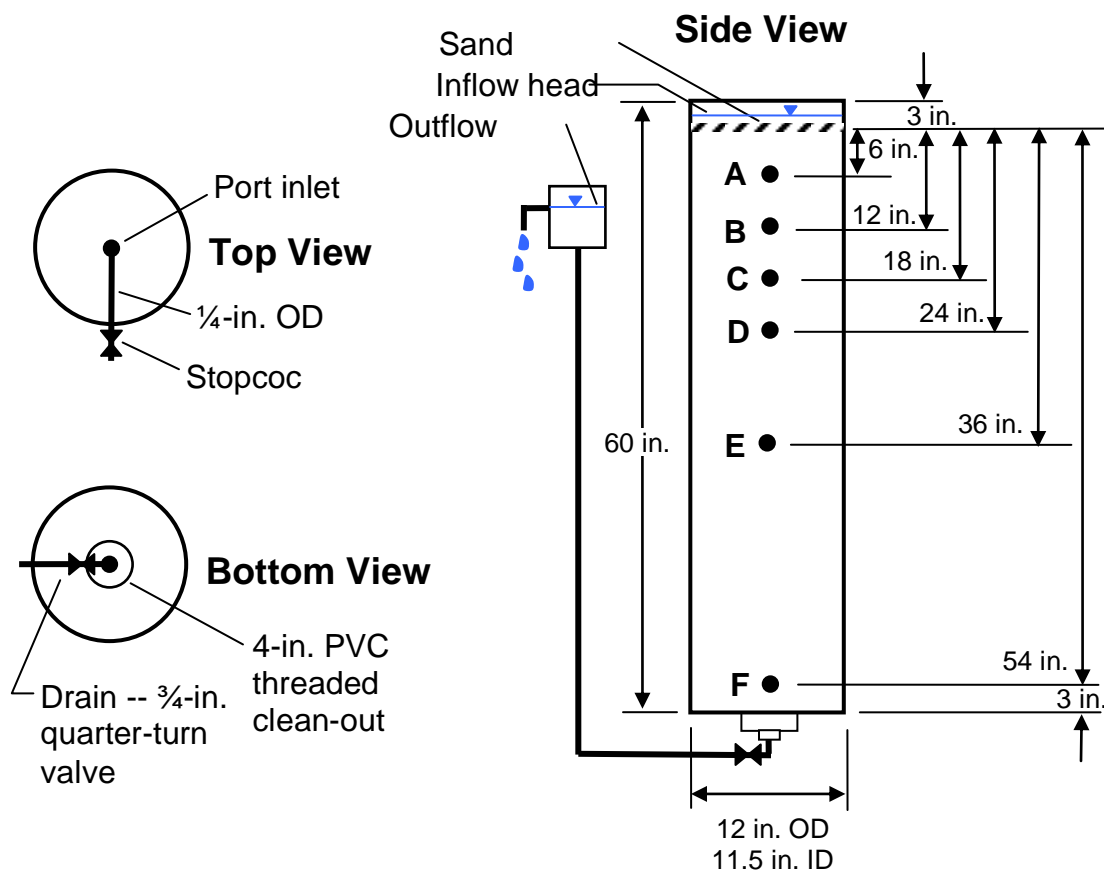


Figure 1. Soil column diagram.

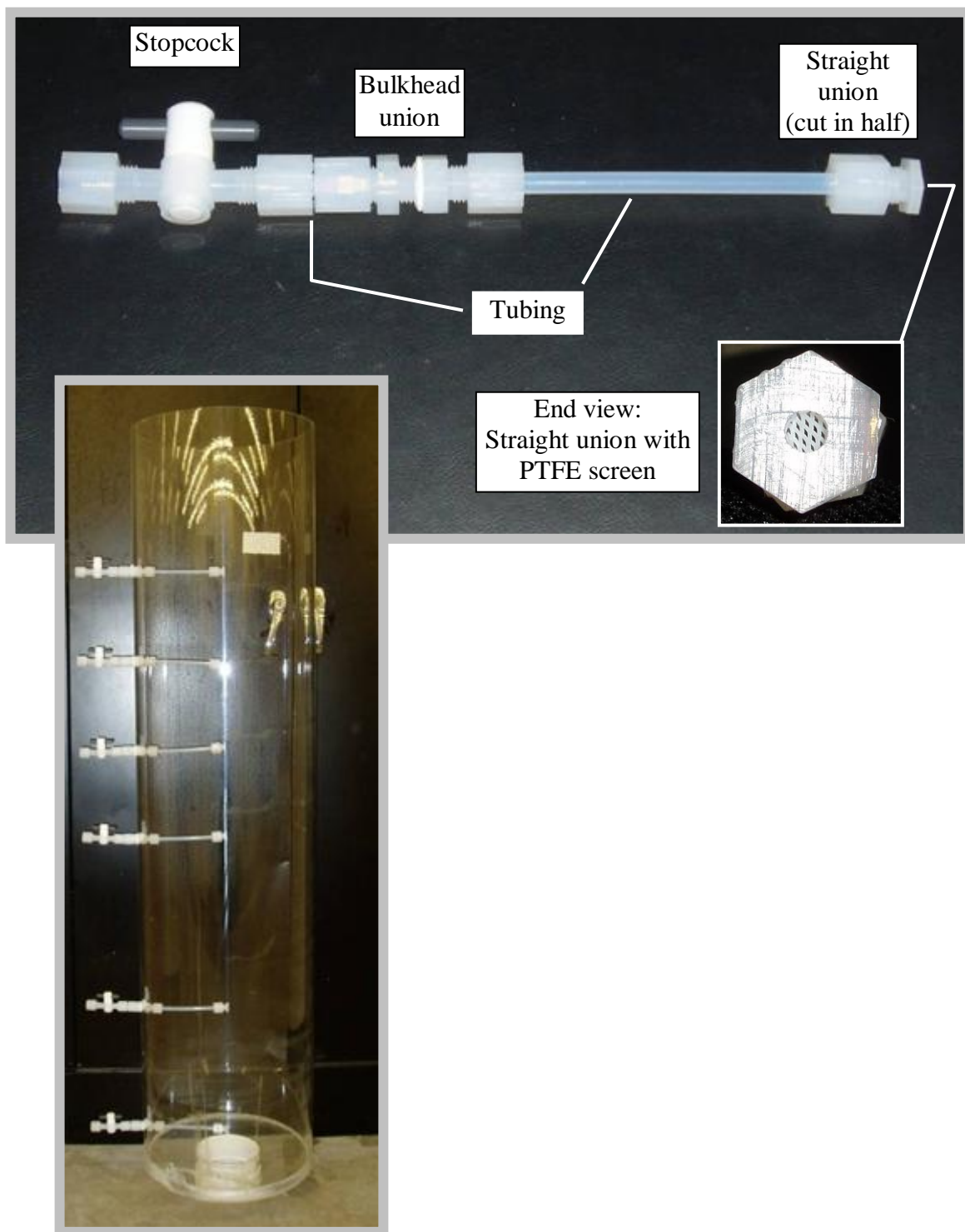


Figure 2. Soil column and sampling port assembly.

The experiments were performed using poorly graded, clean sand (Figure 3). Three columns were packed with a 1-inch thick layer of pea gravel in the bottom and next with the air-dried sand up to 3 inches below the top of each column (Figure 1). Sand was compacted in 6-inch layers to a dry bulk density of approximately 100 pounds per cubic foot (lbs/ft^3) ($1.6 \text{ grams per cubic centimeter, g/cm}^3$). Analysis of a small core packed to a density of 1.56 g/cm^3 indicated a porosity of 0.41 for this sand.

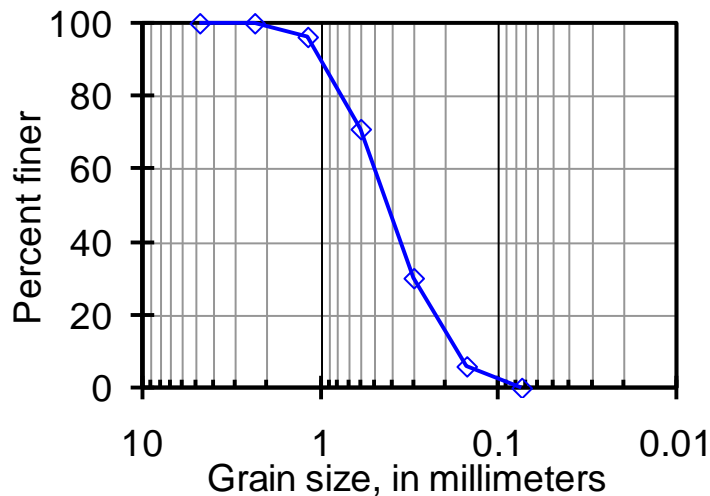


Figure 3. Grain-size distribution of sand used in soil columns.

Columns were saturated in July 2006 with natural stormwater from Pegasus Pond on the campus of the University of Central Florida. Previous testing of Pegasus Pond for microcystin indicated concentrations ranging from a non-detect ($<0.04 \text{ ug/L}$) to 0.16 ug/L in 2005 (Wanielista et al, 2006) and 1.0 ug/L in August 2007. Only natural stormwater was used during all column preconditioning and testing (Figure 4). In February 2007 Column #2 developed a leak. Attempts were made to repair the leak, but were unsuccessful and Column #2 was drained and not used after October 2007. In June 2007, Column #1 developed a leak and partially drained. In September 2007, Column #1 was completely drained, the leak successfully repaired, and the column resaturated. Column #2 has remained saturated since September 2007. Column #3 has remained saturated since July 2006.



Figure 4. Soil columns packed with sand. From the front right column, columns were identified as #1, #2, and #3 in a counter-clockwise direction.



Columns #1 and #3 were operated during both experiments in a gravity-feed manner. A multi-channel peristaltic pump was used to deliver a constant rate of flow to the top of each column (Figure 4). A 1-inch depth of ponded water was maintained by adjusting the outflow head of each column (Figures 1 and 4).

Soil Column Experiments

Two soil column experiments were performed: Experiment #1 commenced February 6, 2008 at 2:10 pm EST; and Experiment #2 commenced August 12, 2008 at 12:30 pm EDT. During the days immediately preceding each experiment, each column was flushed with a minimum of three pore volumes using natural stormwater. Natural stormwater from Pegasus Pond was used during both column experiments. For Experiment #1, columns #1 and #3 were run simultaneously for 75 hours using stormwater spiked to yield a MC-LR concentration of 3.9 ug/L; sampling intervals ranged from 0.5 to 14 hours, and a total of 120 samples were collected. For Experiment #2, columns #1 and #3 were run simultaneously for 10 days using stormwater

spiked to yield a MC-LR concentration of 2.2 ug/L and a cylindrospermopsin concentration of 0.5 ug/L; sampling intervals ranged from 0.5 to 48 hours, and a total of 190 samples were collected. Columns were kept in the dark by wrapping in a tarp and were maintained in a temperature range of 18-22 °C to mimic subsurface conditions.

Samples were collected from each of six sampling ports (labeled A-F) on each column as well as from the ponded water (labeled P) at the top of the column (Figure 1). Samples were immediately filtered upon collection, placed in 40 milliliter (mL) pre-cleaned amber glass vials with PTFE lined septa (EP Scientific Products, Miami, OK) and refrigerated (0-4 °C). After refrigeration, samples were preserved (frozen at -40 °C) within 24 hours of collection. Samples were collected using 30-mL single-use syringes (Becton, Dickson and Company, Franklin Lakes, NJ) and 0.45-micrometer glass-microfiber single-use syringe filters (25mm GD/X, Whatman, Kent, UK).

To assess the presence of cell-bound microcystin in cyanobacterial cells possibly present in the natural stormwater, unfiltered samples of the stormwater were collected. A freeze-thaw process (Graham and Jones, 2007; Graham et al, 2008) was used to lyse the cells, thereby releasing any toxin if present. The sample was frozen, thawed, and agitated; this process was repeated for three cycles. The lysed sample was filtered and analyzed by ELISA as described above.

During both experiments, a thin (1-2 mm thick) layer continually formed on the sand surface with a black organic appearance, presumably a “biofilm” layer, which caused significant head loss across the column. During Experiment #1, the outflow heads of each column were adjusted to operate both columns at an infiltration rate of about 1 inch per hour (in/hr), which is equivalent to a pore-water velocity of 2.4 in/hr for a porosity of 0.41 (Figure 5A). Following Experiment #1 this biofilm layer deteriorated and dissipated as the columns remained stagnant, so that it was no longer visibly intact by the start of Experiment #2. During Experiment #2 the biofilm layer formed again; the outflow heads of each column were adjusted to maintain an infiltration rate of about 1 in/hr for about the first 4 days, after which infiltration decreased dramatically due to substantially clogging caused by the biofilm layer and the inability to lower the outlet head enough to compensate (Figure 5B).

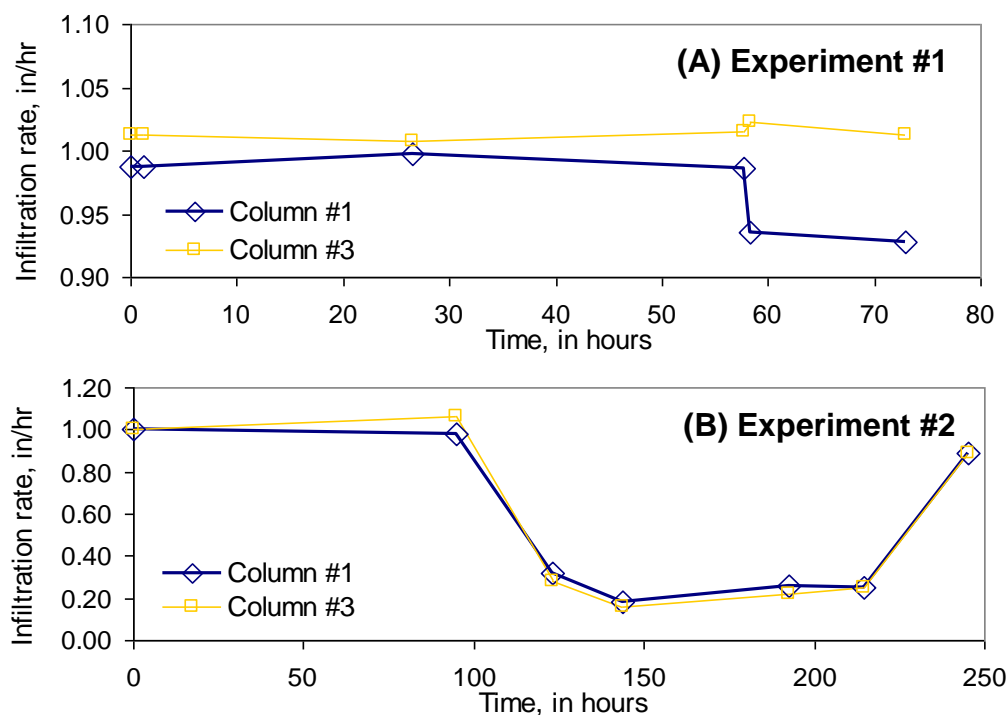


Figure 5. Infiltration rates during (A) Experiment #1; and (B) Experiment #2.

Laboratory Analysis of Microcystin Concentration

Filtered water samples, thawed and brought to room temperature, were quantitatively analyzed using enzyme-linked immunosorbent assay (ELISA) using a VERSAmax Tunable Microplate Reader (Molecular Devices Corporation, Sunnyvale, CA) and commercially available 96-well microplate kits (Microcystin-ADDA Microtiter Plate, Product No. 520011, Abraxis, Warminster, PA). This competitive indirect ELISA was developed from antibodies recognizing the ADDA moiety (an amino acid), which is the common structural feature present in greater than 80 percent of the toxic congeners of microcystins and nodularins (Fischer et al, 2001). Therefore, concentrations derived from this ELISA represent total microcystins plus nodularins expressed as MC-LR equivalents. ELISAs were performed at the University of Central Florida Department of Civil, Environmental, and Construction Engineering Organic Chemistry Laboratory according to the manufacturer's kit instructions (A.M. O'Reilly, U.S. Geological Survey, written commun., 2009). A standard curve relating relative absorbance (absorbance of sample divided by the absorbance of the zero standard at 450 nanometers) to MC-LR concentration was developed for each plate using a four-parameter sigmoid function, which is a generalization of the commonly used log-logit function (Molecular Devices Corporation, 2004, p. 353). All samples were run in duplicate and absorbances averaged before computing the

microcystin concentration via the standard curve. Per the manufacturer's specifications, the detection limit for the ELISA is 0.1 ug/L. Samples with concentrations exceeding 4.7 ug/L (the highest standard used to derive the standard curve was 5 ug/L) were diluted using the diluent supplied with the ELISA kit and reanalyzed.

In order to quantify bias and variability in ELISA results, numerous quality control (QC) samples were run with each plate, including at least one blank and 5-7 control samples of known concentration (0.75, 1.0, and/or 2.0 ug/L). A total of 11 plates were used to analyze all samples from both experiments, resulting in 13 blanks and 72 control samples. Complete QC results are provided in Appendix A. Based on these QC samples, control charts for accuracy and precision were developed (Figure 6). Percent recovery was computed as a measure of accuracy (bias):

$$\text{Percent Recovery} = \frac{\text{Observed Concentration}}{\text{True Value}} \times 100.$$

Relative percent difference (RPD) was computed as a measure of precision (variability):

$$\text{RPD} = \frac{|A - B|}{A + B} \times 200$$

where,

A = Percent recovery from control 1; and
B = Percent recovery from control 2.

Satisfactory accuracy was achieved based on percent recoveries with a mean of 105 % and standard deviation of 13.8 %; satisfactory precision was achieved based on RPDs with a mean of 11 % and standard deviation of 8.1 %. Control charts indicate nearly all control samples were within two standard deviations of the respective mean values (Figure 6).

Microcystin concentrations for 13 blanks were all less than 0.1 ug/L, with a mean of 0.046 ug/L and standard deviation of 0.035 ug/L. A common method for computing the method detection limit (MDL) uses the following equation (Eaton et al, 2005):

$$\text{MDL} = t_{v,\alpha} * s$$

where,

$t_{v,\alpha}$ = one-sided Student's t-value;
v = degrees of freedom;
alpha = significance level; and
s = standard deviation of at least 7 blanks.

Applying this equation for a confidence level of 99 percent (alpha = 0.01) yields a MDL of 0.095 ug/L, which agrees well with the manufacturer's detection limit of 0.1 ug/L. For the ELISA concentrations reported herein, any result less than 0.1 ug/L is reported as 0.00 ug/L. However, it should be noted that these samples might actually have nonzero microcystin concentrations less than the MDL.

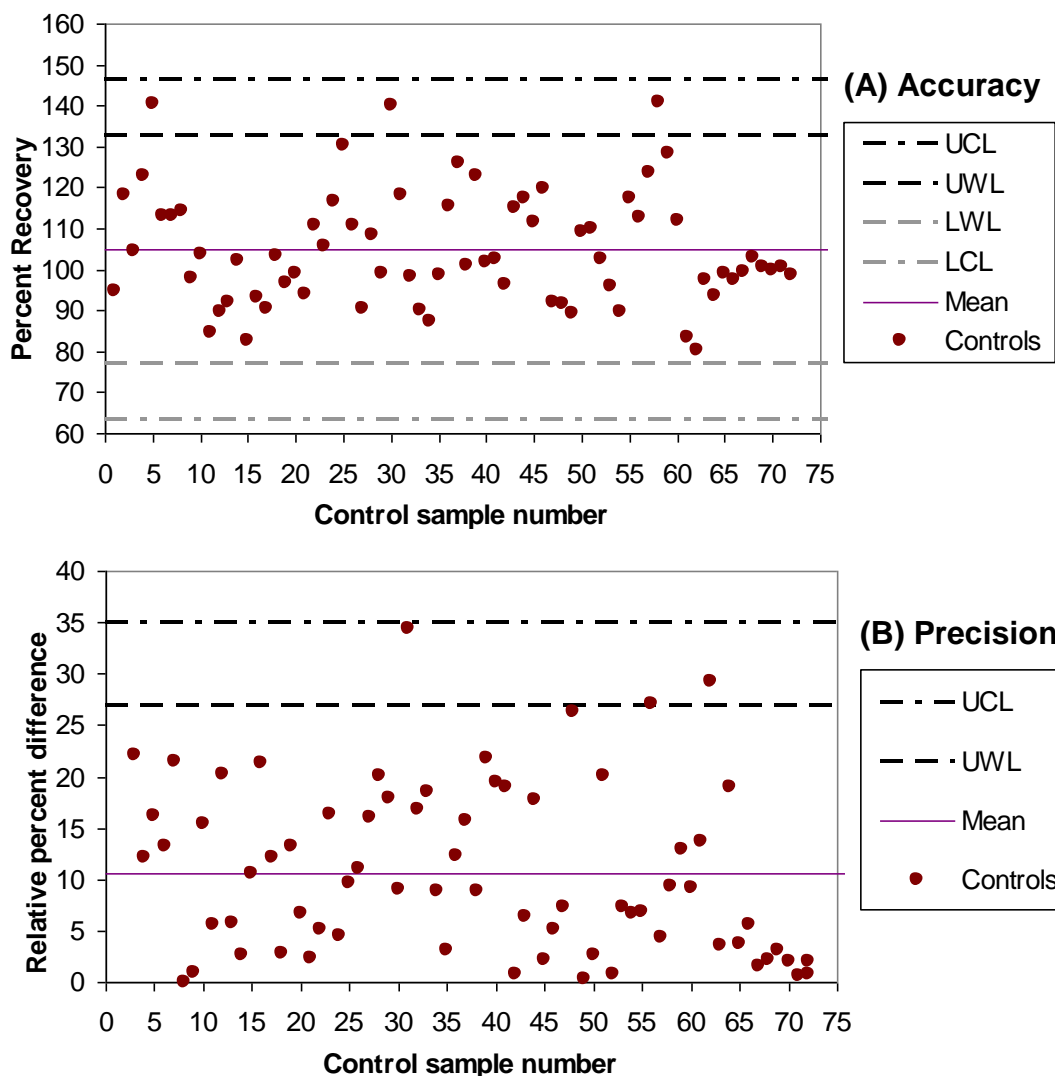


Figure 6. Control charts for (A) accuracy; and (B) precision. UCL, upper control limit (mean + 3 standard deviations); UWL, upper warning limit (mean + 2 standard deviations); LWL, lower warning limit (mean – 2 standard deviations); LCL, lower control limit (mean – 3 standard deviations).

A subset of samples (16) from Experiment #1 was analyzed by the U.S. Geological Survey Organic Geochemistry Research Laboratory (K.A. Loftin, U.S. Geological Survey, written commun., 2008) using liquid chromatography with tandem mass spectrometry (LC/MS/MS) (Dahlman and others, 2003; Spoof and others, 2003) to confirm results by ELISA. LC/MS/MS analyses includes quantification of concentrations for seven microcystin congeners

(LA, LF, LR, LW, LY, RR, and YR) as well as four other cyanotoxins (nodularin, lyngbyatoxin, anatoxin-a, and cylindrospermopsin). LC/MS/MS analyses have a reporting limit of 0.01 ug/L.

Experimental Results

Column experiment results generally are most conveniently expressed in the form of breakthrough curves (BTCs), with concentration plotted as a function of time. To facilitate comparison of BTCs among sampling ports for both experiments, dimensionless BTCs are presented herein. Further details of all results, including dimensional concentration and time variables are presented in Appendix B. Dimensionless concentration (C/C_o) is the ratio of concentration (C) to influent concentration (C_o). A dimensionless measure of water contact time with column media is pore volume (T) (Alvarez and Illman, 2006, p. 330):

$$T = \frac{V}{n A L}$$

where,

V = Volume of water passed through column at time t ;

n = Porosity;

A = Cross-sectional area of column; and

L = Length of media above sampling port inlet.

Results from samples collected from the ponded water in each column are plotted as a function of elapsed time (t), because a “pore volume” obviously has no definition in this situation (Figure 7). Microcystin concentrations remained essentially constant in the ponded water during Experiment #1 in both columns at a value of about 3.9 ug/L (which was used as the value for C_o). During Experiment #2, microcystin concentrations remained approximately constant in the ponded water during the first 5 days in both columns at a value of about 2.2 ug/L (which was used as the value for C_o), after which time concentrations decreased in both columns during the remainder of the experiment. This decrease in concentration may be due to microbial degradation in the ponded water in close proximity to the biofilm layer on the sand surface, which was well formed by the fourth day of the experiment.

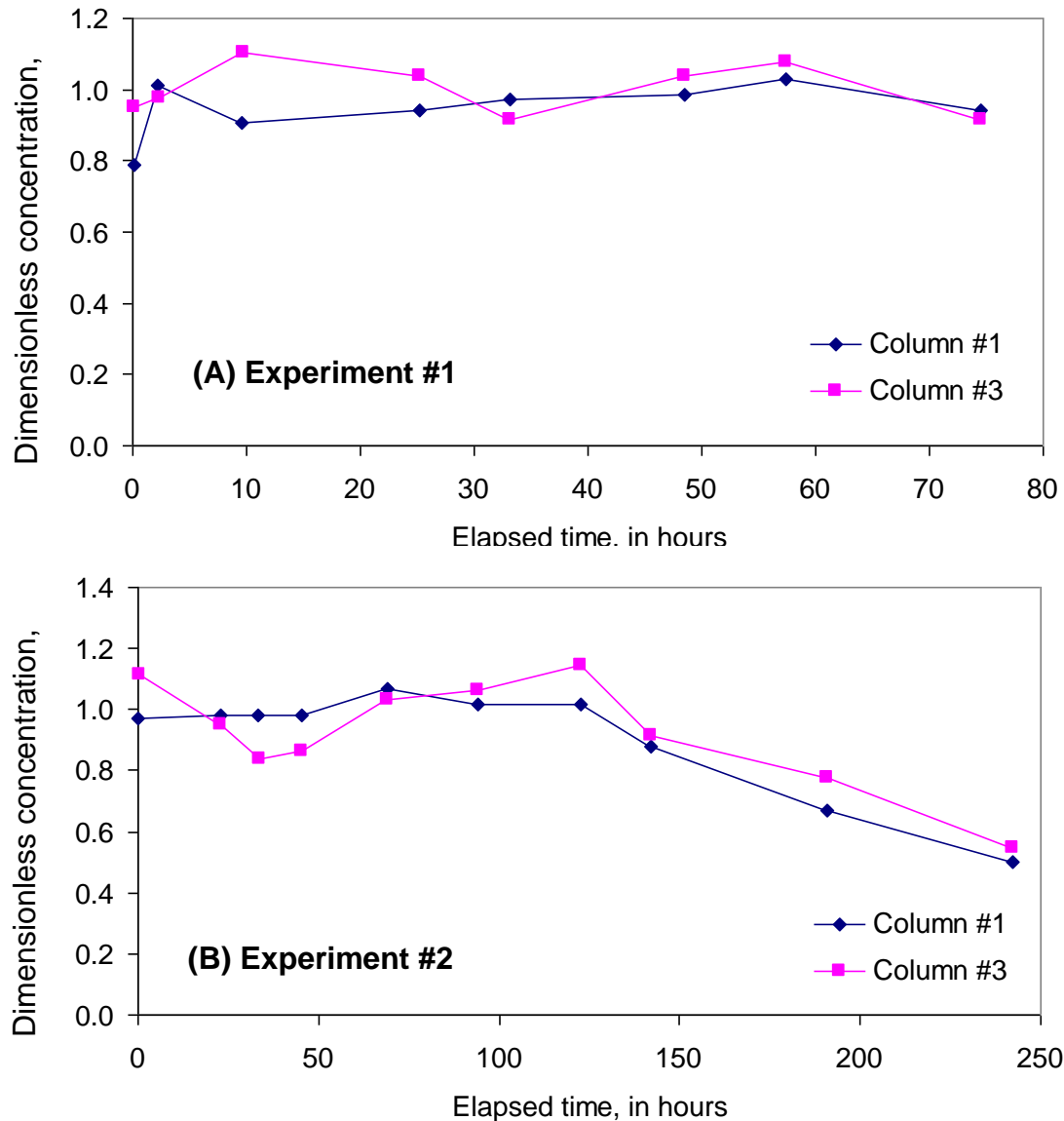


Figure 7. Dimensionless microcystin concentrations in the ponded water at the top of both columns in (A) Experiment #1, and (B) Experiment #2.

BTCs from both columns show generally similar patterns in microcystin concentration (Figures 8 and 9):

- Breakthrough ($C/C_o = 0.5$) that generally occurs at about one pore volume (based on a porosity of 0.41), suggesting sorption processes are limited;
- A continued increase in concentration above the influent concentration after breakthrough; and
- A decline in concentration shortly after complete breakthrough, suggestive of first-order decay at some sampling ports, possibly caused by microbial degradation.

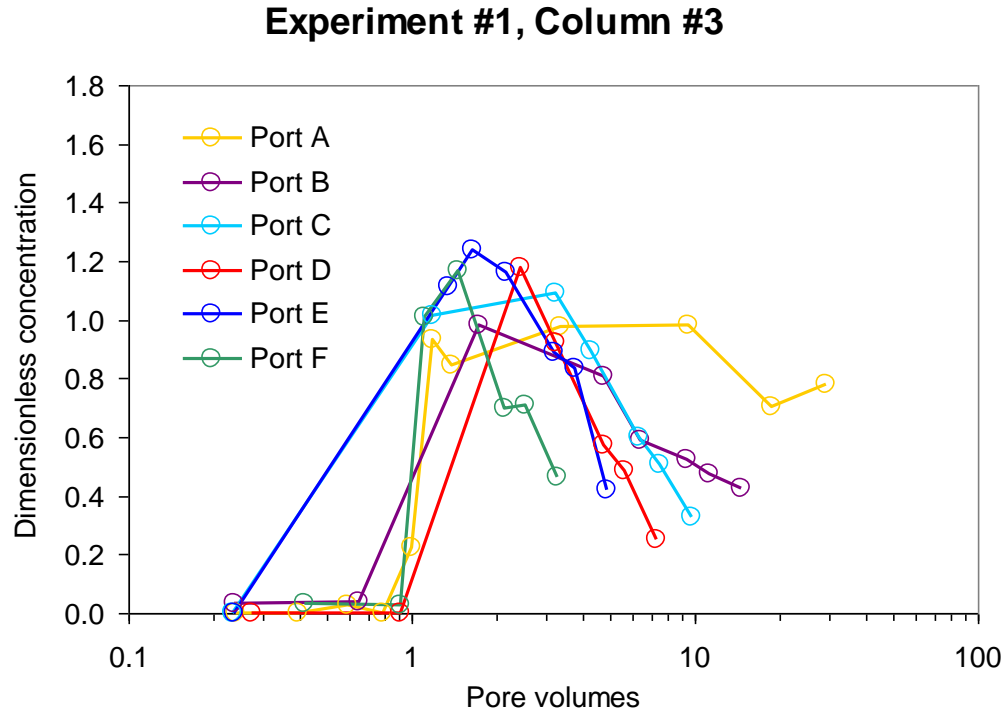
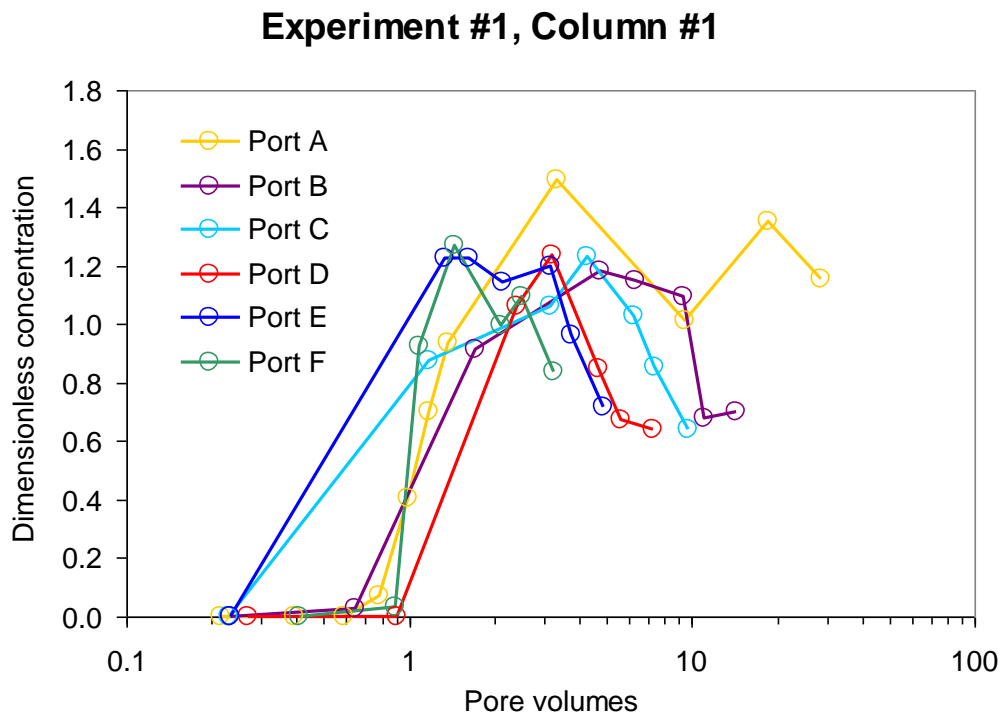
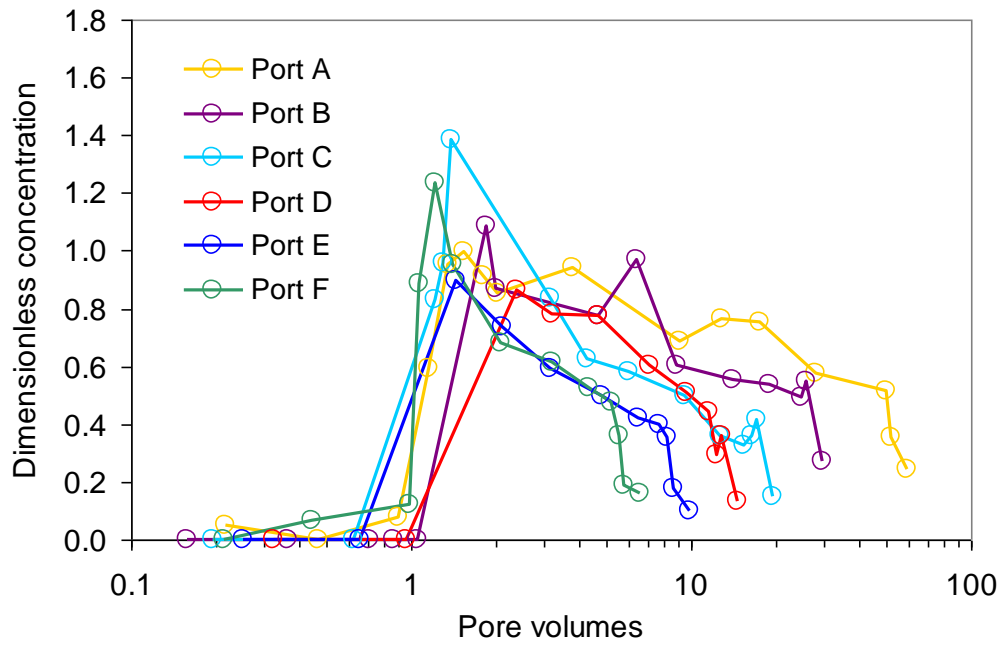


Figure 8. Dimensionless breakthrough curves for Experiment #1.

Experiment #2, Column #1



Experiment #2, Column #3

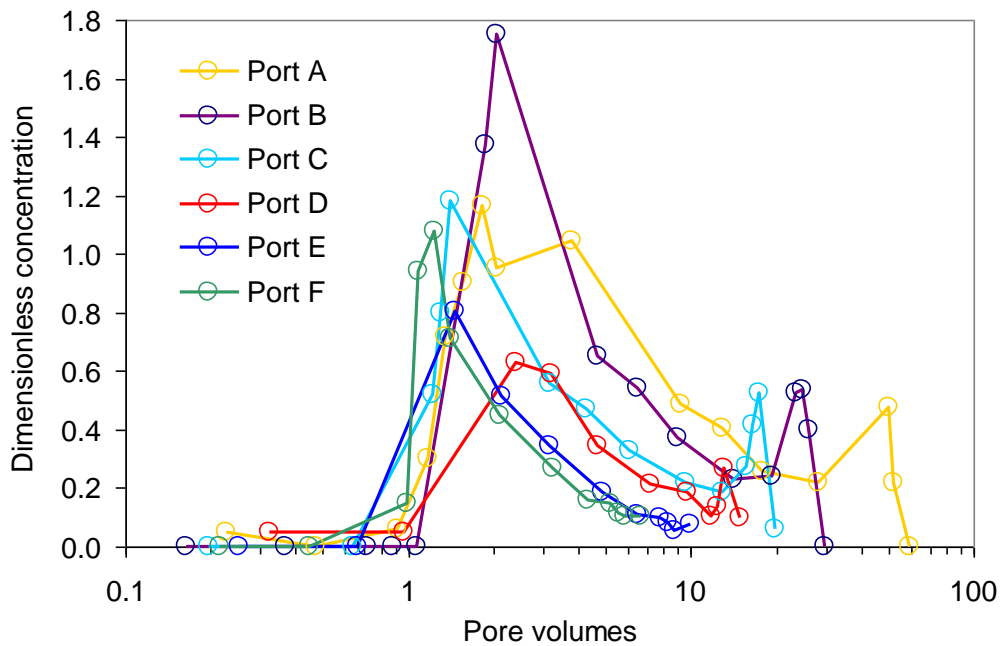


Figure 9. Dimensionless breakthrough curves for Experiment #2.

Reasons for concentrations greater than the influent concentration ($C/C_o > 1$) are not clear, but may be due in part to variability in the ELISA technique. Recall that percent recoveries of control samples indicate that about 95 percent had percent recoveries ranging from 77 to 133 percent (Figure 6A). Most of the peak dimensionless concentrations are below a C/C_o value of 1.3, thus falling with the range of accuracy expected for these ELISAs. No apparent “source” of microcystin was present in the columns that could cause an increase in concentration above the influent concentration: initial samples collected prior to breakthrough indicate the microcystin concentrations were very low (0.12 ug/L) or less than the MDL at all sampling ports for both experiments; and analysis of lysed whole-water samples from both experiments indicated that cell-bound microcystin was negligible or nonexistent.

Despite the variability inherent in ELISA analyses, the *shapes* of the BTCs are consistent, indicating little initial change in microcystin concentrations and relatively conservative transport at breakthrough followed by substantial declines in concentration. This is especially evident during Experiment #2, which was of longer duration (10 days). The percent change in final concentration at the end of each experiment for each sampling port relative to the initial concentration illustrates this substantial reduction in microcystin concentrations (Figure 10). These results are qualitatively consistent with those reported by other researchers. Soil column studies performed by Lahti et al (1996, 1998) indicated that decreases in microcystin concentration occurred from about 3 to 9 days after loading commenced. Based on batch experiments, Miller and Fallowfield (2001) reported that complete toxin removal occurred within 10-16 days for certain soils. Effectiveness of slow sand filtration for removal of microcystins from drinking water was investigated by Grützmacher et al (2002a). They used a full-scale, sand filtration system and demonstrated that microbial degradation substantially reduced the concentrations of both intracellular and extracellular microcystins under moderate temperatures and with a biofilm established by previous contact with water containing microcystins.

For Experiment #2, the decrease in influent concentration in both columns (Figure 7B) likely affected sample concentrations later in the experiment. This decrease in influent microcystin concentration begins about 122 hours after the start of the experiment, which corresponds to the following pore volumes for each sampling port: port A, 46; port B, 23; port C, 15; port D, 15; port E, 8.0; and port F, 5.3. Microcystin concentrations of samples collected after these noted pore volumes probably are lower than what would have occurred if the influent concentration had remained constant. The sharp declines at the tail end of many BTCs (Figure 9) likely are due, at least in part, to the decline in influent concentration. Substantial declines in all BTCs, however, also occurred before the decline in influent concentration.

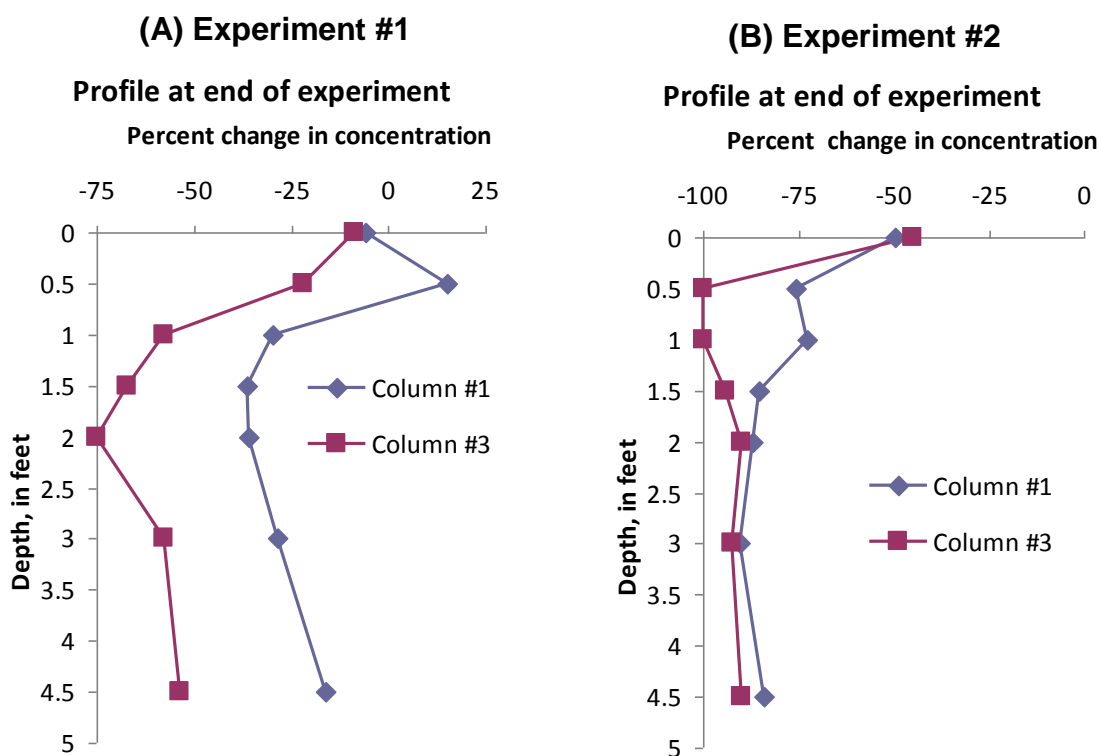


Figure 10. Percent change in microcystin concentration (relative to influent concentration) by the end of (A) Experiment #1, and (B) Experiment #2.

In order to further investigate microcystin loss and as a quality assurance measure, a mass balance was computed for each sampling port of each column. Mass loss for the entire duration of the experiment was computed as follows:

$$\text{Mass Loss} = \text{Mass In} - \text{Mass Out} - \text{Change in Storage}$$

The control volume for the mass balance for each sampling port was the entire volume of column between the sand surface and the respective port inlet. Mass-In quantities were computed by integrating the influent concentration curves (Figure 7) and Mass-Out quantities were computed by integrating the BTCs (Figures 8 and 9). The numerical integration was implemented using the trapezoidal rule. For ports with sparse sampling during breakthrough (see ports C, D, and E for Experiment #1 in Figure 8 and ports D and E for Experiment #2 in Figure 9), the Mass-Out quantities obtained via this integration probably are in error to some degree. The Change-in-Storage quantities were computed based on the final concentrations for the BTCs; the initial concentrations were assumed to be zero because all the initial concentrations for the BTCs were very close to or less than the ELISA MDL. Mass loss computed as a percentage of mass input shows microcystin mass losses for both columns except Column #1 in Experiment #1. Column

#1 in Experiment #1 indicates little change in microcystin mass, considering that the variability indicated in Figure 11A is likely within the range of variability of ELISA analyses. In fact, in both experiments Column #1 had lower mass loss percentages (Figure 11) and higher final microcystin concentrations (Figure 10). The reason for this is unknown, but may be related to the draining of Column #1 for repair about three months prior to Experiment #1, as previously described. Nevertheless, during Experiment #2 both columns experienced substantial mass losses ranging from 37 to 76 % (Figure 11B), which likely can be attributed to the longer duration of Experiment #2 (10 days) compared to Experiment #1 (3 days).

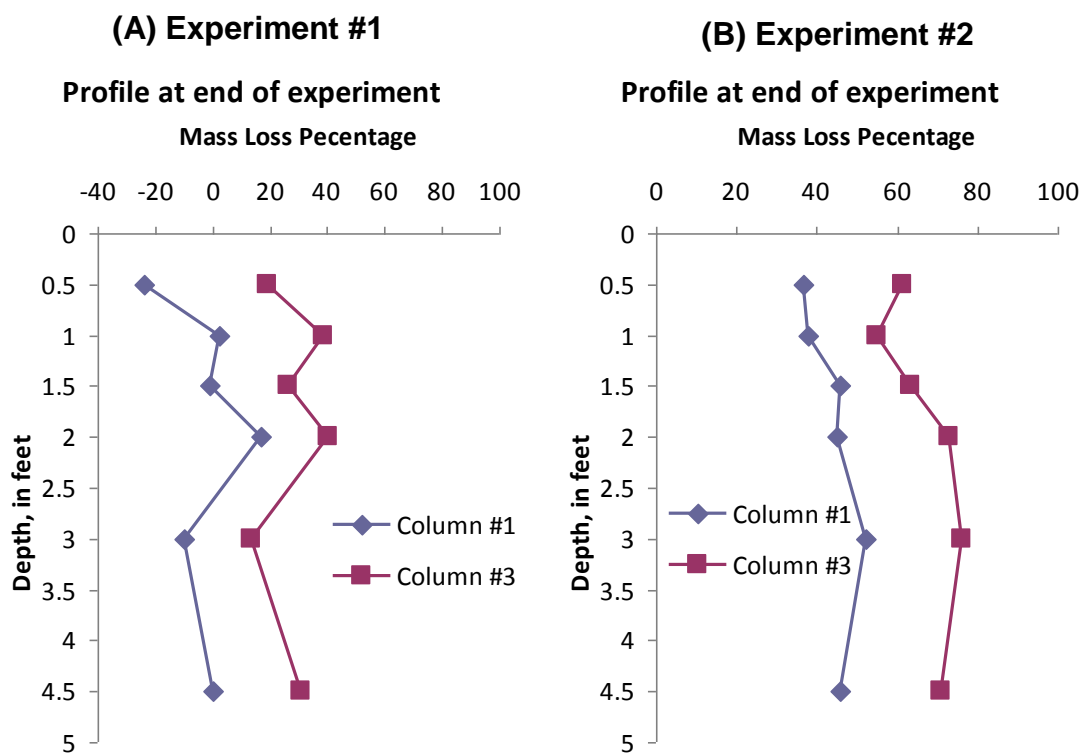


Figure 11. Microcystin mass loss percentages (relative to total mass input) by the end of (A) Experiment #1, and (B) Experiment #2.

A comparison of ELISA and LC/MS/MS results from 16 samples from Experiment #1 indicate that concentrations derived from ELISA generally were greater than those derived from LC/MS/MS (Figure 12). A complete listing of all LC/MS/MS results is presented in Appendix C. These differences may be due, in part, to the congener specific nature of LC/MS/MS and the largely congener independent nature of the ELISA used in this study. If there were other microcystin (or nodularin) congeners present in the natural stormwater, other than the eight congeners analyzed by LC/MS/MS, the concentrations determined from ELISA would be larger

due to the high cross-reactivity of the ELISA with toxic congeners containing the ADDA moiety. LC/MS/MS results indicate all algal toxins tested, other than the MC-LR congener, were below the detection limit of 0.01 ug/L. ELISA results indicate that the spiked natural stormwater sampled from the ponded water at the top of the columns averaged 3.9 ug/L during Experiment #1, about 18 percent higher than that derived from dilution calculations of 3.3 ug/L, which is well within the range of variability reported for the ELISA analyses (Figure 6). This suggests that concentrations of microcystin congeners other than MC-LR were small or nonexistent during Experiment #1. Despite these apparent disparities between concentration magnitudes, LC/MS/MS and ELISA results for sequential samples collected from ports B and D all indicate substantial downward trends (Figure 13).

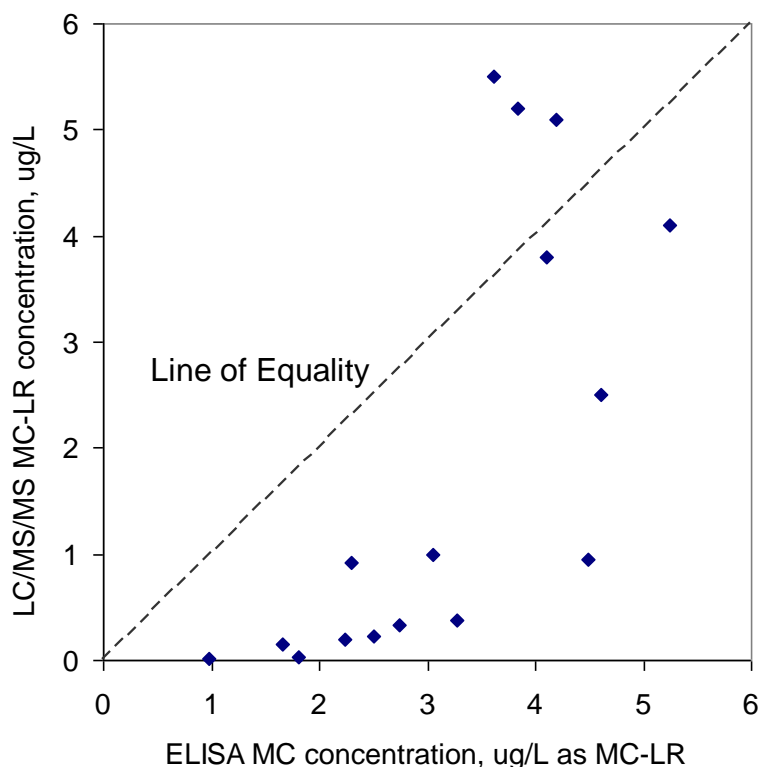


Figure 12. Comparison of LC/MS/MS and ELISA results for 16 samples from Experiment #1.

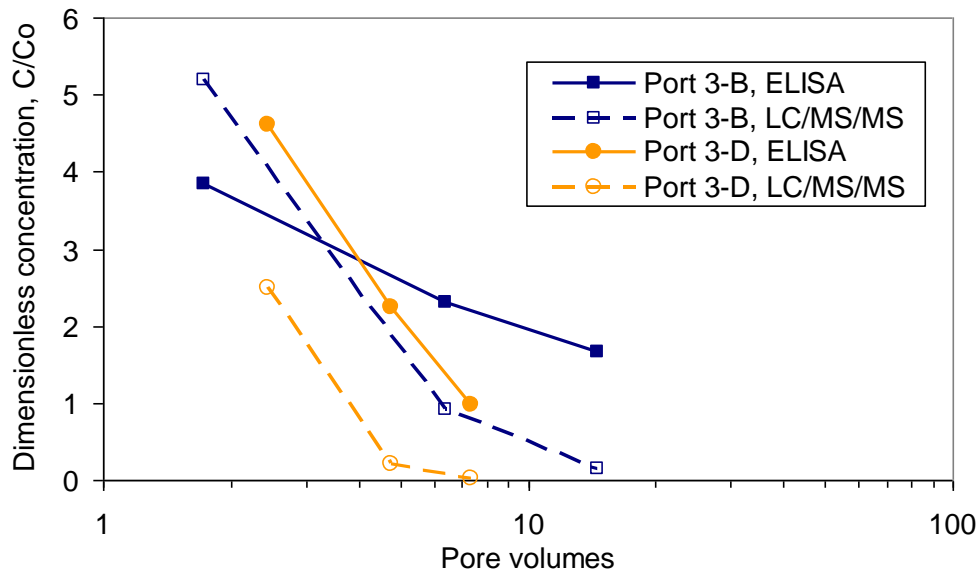


Figure 13. Comparison of LC/MS/MS and ELISA results for ports B and D from Experiment #1.

Conclusions

Results of two soil column experiments indicate the potential for substantial removal of the cyanotoxin microcystin during saturated flow through sand. During the first (3-day) experiment, microcystin removal efficiencies (based on initial and final concentrations) for each column averaged approximately 20 percent for Column #1 and 60 percent for Column #3, and mass removal efficiencies based on mass balances averaged approximately 0 percent for Column #1 and 30 percent for Column #3 (Figures 10 and 11). During the second (10-day) experiment, microcystin removal efficiencies (based on initial and final concentrations) for each column averaged approximately 80 percent for Column #1 and 90 percent for Column #3, and mass removal efficiencies based on mass balances averaged approximately 40 percent for Column #1 and 70 percent for Column #3 (Figures 10 and 11). Breakthrough curves indicate relatively conservative transport of microcystin at breakthrough, suggesting sorption processes are limited, followed by substantial declines in concentration, suggesting kinetic reaction processes are important and possibly caused by microbial degradation (Figures 8 and 9). Microcystin losses generally increased with depth up to 2 ft and remained relatively constant at greater depths (Figures 10 and 11). During the 10-day experiment, substantial losses occurred in the upper 0.5 ft where microcystin concentration was reduced below the ELISA detection limit in one column (Figure 10B), although this probably is due in part to the microcystin degradation occurring in the ponded water (Figure 7B). The longer duration of the second experiment probably contributed to the greater microcystin loss by allowing additional time for microbial acclimation and growth. Microbial degradation likely can be attributed mostly to the biofilm layer that formed on the sand surface, thus causing the large microcystin losses in the upper parts of the columns, which might also have contributed to the microcystin losses in the ponded water above

the biofilm layer. Grützmacher et al (2002a) reported similar results during slow sand filtration (2.6-ft thick filter bed), noting not only negligible adsorption but also the formation of a “schmutzdecke” postulated to be the location of most of the microcystin degrading biological activity. However, examination of the BTCs for the second experiment indicates generally lower concentrations in deeper sampling ports, suggesting additional removal capacity is afforded by the thicker filter bed (Figure 9). Based on the soil and water quality conditions used in the experimental setup, a sand filter bed ranging from 2 to 4 feet in thickness is sufficient for effective microcystin removal.

References

- Abbott, G.M., Landsberg, J.H., Reich, A.R., Steidinger, K.A., Ketchen, S., and Blackmore, C., 2009, Resource guide for public health response to harmful algal blooms in Florida: Fish and Wildlife Research Institute Technical Report TR-14, 132 p.
- Alvarez, P.J.J., and Illman, W.A., 2006, Bioremediation and Natural Attenuation: Process Fundamentals and Mathematical Models: Hoboken, NJ, John Wiley & Sons, Inc, 609 p.
- Burns, J., Williams, C., and Chapman, A., 2002, Cyanobacteria and their toxins in Florida surface waters, *in* Johnson, D., and Harbison, R.D. (eds.), Proceedings of Health Effects of Exposure to Cyanobacteria Toxins: State of the Science, Aug. 13-14, 2002, Sarasota, Fla., p. 16-21.
- Dahlmann, J., Budakowski, W.R., Luckas, B., 2003, Liquid chromatography-electrospray ionization-mass spectrometry based method for the simultaneous determination of algal and cyanobacterial toxins in phytoplankton from marine waters and lakes followed by tentative structural elucidation of microcystins: *Journal of Chromatography*, v. 994, p. 45–57.
- Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E., and Franson, M.A.H. (eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed.: Washington D.C., American Public Health Association.
- Graham, J.L., and Jones, J.R., 2007, Microcystin distribution in physical size class separations of natural plankton communities: *Lake and Reservoir Management*, v. 23, no. 2, p. 161–168.
- Graham, J.L., Loftin, K.A., Ziegler, A.C., and Meyer, M.T., 2008, Guidelines for design and sampling for cyanobacterial toxin and taste-and-odor studies in lakes and reservoirs: U.S. Geological Survey Scientific Investigations Report 2008–5038, 39 p.
- Grützmacher, G., Böttcher, G., Chorus, I., and Bartel, H., 2002a, Removal of microcystins by slow sand filtration: *Environmental Toxicology*, v. 17, no. 4, p. 386-394.
- Grützmacher, G., Böttcher, G., Chorus, I., Knappe, A., and Pekdeger, A., 2002b, Cyanobacterial toxins in bank filtered water from Lake Wannsee, Berlin, *in* P.J. Dillon (ed.), Management of Aquifer Recharge for Sustainability, Proceedings of the Fourth International Symposium on Artificial Recharge, Adelaide, Australia, Balkema Press, p. 175-179.
- Lahti, K., Kilponen, J., Kivimäki, A.L., Erkomaa, K., and Sivonen, K., 1996, Removal of cyanobacteria and their hepatotoxins in soil and sediment columns, *in* Kivimäki, A.L., and Suokko, T. (eds.), Proceedings of an International Symposium on Artificial Recharge of Groundwater, Helsinki, Finland, Nordic Hydrol. Prog. Series, NHP Report No. 38, p. 187-195.

- Lahti, K., Vaitomaa, J., Kivimäki, A.-L., and Sivonen, K., 1998, Fate of cyanobacterial hepatotoxins in artificial recharge of groundwater and in bank filtration, in J.H. Peters and others (eds.), Artificial recharge of groundwater, Balkema, Rotterdam, p. 211-216.
- Miller, M.J, and Fallowfield, H.J., 2001, Degradation of cyanobacterial hepatotoxins in batch experiments: Water Science and Technology, v. 43, no. 12, p. 229-232.
- Molecular Devices Corporation, 2004 SoftMax Pro User Manual: SoftMax Pro Software Version 4.7 for Macintosh and Windows: Sunnyvale, CA, 704 p.
- Spoof, L., Vesterkvist, P., Lindholm, T., Meriluoto, J., 2003, Screening for cyanobacterial hepatotoxins, microcystins and nodularin in environmental water samples by reversed-phase liquid chromatography-electrospray ionization mass spectrometry: Journal of Chromatography, v. 1020, p. 105–119.
- Svrcek, Clark, and Smith, D.W., 2004, Cyanobacteria toxins and the current state of knowledge on water treatment options: a review: Journal of Environmental Engineering and Science, v. 3, p. 155-185.
- Wanielista, M.P., Miller, R., and Pernezny, B., 2006, Stormwater irrigation volume II: Cyanobacteria counts with toxin concentrations in stormwater ponds and after biofiltration to reduce health risks: Stormwater Management Academy, Univ. of Central Florida, 108 p.

Appendix A – ELISA Results, Quality Control

Table A.1. Quality control samples – Positive controls of known concentration.

Control Number	Run Date	ID	Known Concentration (ug/L)	Percent Recovery	Relative Percent Difference
1	3/20/2008	pc	0.75	95	--
2	3/20/2008	LFB1	1.00	118	22
3	3/20/2008	CCV	2.00	105	12
4	3/20/2008	CCV	2.00	123	16
5	3/20/2008	CCV	2.00	141	13
6	3/20/2008	CCV	2.00	113	22
7	3/20/2008	CCV	2.00	113	0
8	5/1/2008	pc	0.75	114	1
9	5/1/2008	LFB	1.00	98	15
10	5/1/2008	LFB	1.00	104	6
11	5/1/2008	CCV	2.00	85	20
12	5/1/2008	CCV	2.00	90	6
13	5/1/2008	CCV	2.00	92	3
14	5/1/2008	CCV	2.00	102	11
15	5/2/2008	pc	0.75	82	21
16	5/2/2008	LFB	1.00	93	12
17	5/2/2008	CCV	2.00	91	3
18	5/2/2008	CCV	2.00	103	13
19	5/2/2008	CCV	2.00	97	7
20	5/2/2008	CCV	2.00	99	2
21	5/2/2008	CCV	2.00	94	5
22	5/8/2008	pc	0.75	111	16
23	5/8/2008	LFB	1.00	106	5
24	5/8/2008	CCV	2.00	117	10
25	5/8/2008	CCV	2.00	130	11
26	5/8/2008	CCV	2.00	111	16
27	1/7/2009	pc	0.75	91	20
28	1/7/2009	CCV	1.00	108	18
29	1/7/2009	CCV	1.00	99	9
30	1/7/2009	CCV	1.00	140	34
31	1/7/2009	CCV	1.00	118	17
32	1/7/2009	CCV	1.00	98	19
33	1/13/2009	pc	0.75	90	9
34	1/13/2009	CkS	2.00	87	3
35	1/13/2009	CCV	1.00	99	12
36	1/13/2009	CCV	1.00	115	16
37	1/13/2009	CCV	1.00	126	9
38	1/13/2009	CCV	1.00	101	22
39	1/13/2009	CCV	1.00	123	20

Control Number	Run Date	ID	Known Concentration (ug/L)	Percent Recovery	Relative Percent Difference
40	1/14/2009	pc	0.75	102	19
41	1/14/2009	CkS	2.00	102	1
42	1/14/2009	CCV	1.00	96	6
43	1/14/2009	CCV	1.00	115	18
44	1/14/2009	CCV	1.00	117	2
45	1/14/2009	CCV	1.00	112	5
46	1/14/2009	CCV	1.00	120	7
47	1/15/2009	pc	0.75	92	26
48	1/15/2009	CkS	2.00	92	0
49	1/15/2009	CCV	1.00	89	3
50	1/15/2009	CCV	1.00	109	20
51	1/15/2009	CCV	1.00	110	1
52	1/15/2009	CCV	1.00	102	7
53	1/15/2009	CCV	1.00	96	7
54	1/16/2009	pc	0.75	90	7
55	1/16/2009	CkS	2.00	118	27
56	1/16/2009	CCV	1.00	113	4
57	1/16/2009	CCV	1.00	124	9
58	1/16/2009	CCV	1.00	141	13
59	1/16/2009	CCV	1.00	128	9
60	1/16/2009	CCV	1.00	112	14
61	1/22/2009	pc	0.75	83	29
62	1/22/2009	CkS	2.00	80	4
63	1/22/2009	CCV	1.00	97	19
64	1/22/2009	CCV	1.00	94	4
65	1/22/2009	CCV	1.00	99	6
66	1/22/2009	CCV	1.00	98	2
67	8/13/2009	pc	0.75	100	2
68	8/13/2009	CkS	2.00	103	3
69	8/13/2009	CCV	1.00	101	2
70	8/13/2009	CCV	1.00	100	1
71	8/13/2009	CCV	1.00	101	1
72	8/13/2009	CCV	1.00	99	2

Table A.2. Quality control samples – Matrix spikes.

Run Date	ID	Matrix Concentration (ug/L)	Spike Concentration (ug/L)	Percent Recovery	Matrix Sample Description
3/20/2008	Mspk	0.06	1.00	76	Experiment #1, Sample 1-A-1
1/16/2009	Mspk	1.04	1.00	103	Experiment #2, Sample 1-F-10

Table A.3. Quality control samples – Blanks.

Run Date	ID	ELISA Concen- tration (ug/L)
3/20/2008	ND	0.030
3/20/2008	B-ss	0.010
3/20/2008	B-eq	0.032
5/1/2008	ND	0.000
5/2/2008	ND	0.085
5/8/2008	ND	0.080
1/7/2009	ND	0.000
1/13/2009	ND	0.088
1/14/2009	ND	0.055
1/15/2009	ND	0.065
1/16/2009	ND	0.088
1/22/2009	ND	0.063
8/13/2009	ND	0.000

Table A.4. Standard curve coefficient of determination (r^2) and the percent recovery for the 0.75 ug/L positive control supplied with the kit.

Run Date	r^2	Percent Recovery
3/20/2008	1.000	95
5/1/2008	0.994	114
5/2/2008	0.999	82
5/8/2008	0.993	111
1/7/2009	1.000	91
1/13/2009	1.000	90
1/14/2009	1.000	102
1/15/2009	1.000	92
1/16/2009	1.000	90
1/22/2009	1.000	83
8/13/2009	0.999	100

Appendix B – ELISA Results, Column Experiments

NOTE: ELISA concentrations are reported to the nearest 0.01 ug/L; however, concentrations should not be interpreted beyond two significant digits.

Table B.1. Microcystin concentrations analyzed with ELISA for Column Experiment #1.

----- COLUMN #1 -----						----- COLUMN #3 -----					
Date	Time (hrs)	Pore Vol-umes	ID	C (ug/L)	C/Co	Date	Time (hrs)	Pore Vol-umes	ID	C (ug/L)	C/Co
2/6/08 14:16	0.10	--	1P-1	3.07	0.788	2/6/08 14:19	0.15	--	3P-1	3.70	0.949
2/6/08 16:25	2.25	--	1P-2	3.95	1.013	2/6/08 16:28	2.30	--	3P-2	3.80	0.976
2/6/08 23:48	9.63	--	1P-3	3.54	0.909	2/6/08 23:52	9.70	--	3P-3	4.30	1.102
2/7/08 15:19	25.15	--	1P-4	3.67	0.942	2/7/08 15:21	25.18	--	3P-4	4.05	1.039
2/7/08 23:19	33.15	--	1P-5	3.79	0.971	2/7/08 23:22	33.20	--	3P-5	3.56	0.913
2/8/08 14:37	48.45	--	1P-6	3.84	0.984	2/8/08 14:41	48.52	--	3P-6	4.05	1.038
2/8/08 23:30	57.33	--	1P-7	4.02	1.030	2/8/08 23:32	57.37	--	3P-7	4.20	1.077
2/9/08 16:42	74.53	--	1P-8	3.67	0.941	2/9/08 16:44	74.57	--	3P-8	3.56	0.913
2/6/08 14:43	0.55	0.22	1A-1	0.00	0.000	2/6/08 14:46	0.60	0.24	3A-1	0.00	0.000
2/6/08 15:10	1.00	0.39	1A-2	0.00	0.000	2/6/08 15:10	1.00	0.40	3A-2	0.00	0.000
2/6/08 15:40	1.50	0.59	1A-3	0.00	0.000	2/6/08 15:40	1.50	0.60	3A-3	0.11	0.027
2/6/08 16:10	2.00	0.78	1A-4	0.28	0.072	2/6/08 16:10	2.00	0.79	3A-4	0.00	0.000
2/6/08 16:42	2.53	0.99	1A-5	1.58	0.404	2/6/08 16:42	2.53	1.00	3A-5	0.88	0.225
2/6/08 17:10	3.00	1.18	1A-6	2.74	0.702	2/6/08 17:10	3.00	1.19	3A-6	3.64	0.933
2/6/08 17:40	3.50	1.37	1A-7	3.64	0.933	2/6/08 17:40	3.50	1.39	3A-7	3.29	0.844
2/6/08 22:40	8.50	3.34	1A-8	5.82	1.494	2/6/08 22:40	8.50	3.37	3A-8	3.81	0.978
2/7/08 14:06	23.93	9.39	1A-9	3.95	1.012	2/7/08 14:06	23.93	9.49	3A-9	3.82	0.980
2/8/08 13:27	47.28	18.55	1A-10	5.27	1.352	2/8/08 13:27	47.28	18.76	3A-10	2.74	0.702
2/9/08 15:23	73.22	28.73	1A-11	4.50	1.153	2/9/08 15:23	73.22	29.04	3A-11	3.05	0.781
2/6/08 15:21	1.18	0.23	1B-1	0.00	0.000	2/6/08 15:21	1.18	0.23	3B-1	0.12	0.032
2/6/08 17:28	3.30	0.65	1B-2	0.10	0.027	2/6/08 17:28	3.30	0.65	3B-2	0.14	0.036
2/6/08 22:54	8.73	1.71	1B-3	3.57	0.916	2/6/08 22:54	8.73	1.73	3B-3	3.84	0.984
2/7/08 14:17	24.12	4.73	1B-4	4.60	1.180	2/7/08 14:17	24.12	4.78	3B-4	3.15	0.809
2/7/08 22:27	32.28	6.33	1B-5	4.48	1.148	2/7/08 22:27	32.28	6.40	3B-5	2.30	0.589
2/8/08 13:36	47.43	9.31	1B-6	4.28	1.097	2/8/08 13:35	47.42	9.41	3B-6	2.04	0.524
2/8/08 22:42	56.53	11.09	1B-7	2.65	0.681	2/8/08 22:42	56.53	11.21	3B-7	1.84	0.473
2/9/08 15:40	73.50	14.42	1B-8	2.74	0.703	2/9/08 15:40	73.50	14.58	3B-8	1.66	0.425
2/6/08 15:56	1.77	0.23	1C-1	0.00	0.000	2/6/08 15:56	1.77	0.23	3C-1	0.00	0.000
2/6/08 23:08	8.97	1.17	1C-2	3.41	0.876	2/6/08 23:08	8.97	1.19	3C-2	3.96	1.016
2/7/08 14:29	24.32	3.18	1C-3	4.14	1.061	2/7/08 14:29	24.32	3.22	3C-3	4.25	1.089
2/7/08 22:37	32.45	4.24	1C-4	4.79	1.229	2/7/08 22:37	32.45	4.29	3C-4	3.49	0.895
2/8/08 13:51	47.68	6.24	1C-5	4.02	1.031	2/8/08 13:51	47.68	6.31	3C-5	2.33	0.597
2/8/08 22:52	56.70	7.42	1C-6	3.33	0.854	2/8/08 22:52	56.70	7.50	3C-6	1.98	0.507
2/9/08 15:54	73.73	9.64	1C-7	2.49	0.638	2/9/08 15:54	73.73	9.75	3C-7	1.28	0.327

----- COLUMN #1 -----						----- COLUMN #3 -----					
Date	Time (hrs)	Pore Vol-umes	ID	C (ug/L)	C/Co	Date	Time (hrs)	Pore Vol-umes	ID	C (ug/L)	C/Co
2/6/08 16:54	2.73	0.27	1D-1	0.00	0.000	2/6/08 16:55	2.75	0.27	3D-1	0.00	0.000
2/6/08 23:23	9.22	0.90	1D-2	0.00	0.000	2/6/08 23:23	9.22	0.91	3D-2	0.00	0.000
2/7/08 14:44	24.57	2.41	1D-3	4.13	1.060	2/7/08 14:44	24.57	2.44	3D-3	4.60	1.180
2/7/08 22:47	32.62	3.20	1D-4	4.82	1.237	2/7/08 22:47	32.62	3.23	3D-4	3.59	0.920
2/8/08 14:02	47.87	4.70	1D-5	3.30	0.847	2/8/08 14:02	47.87	4.75	3D-5	2.24	0.574
2/8/08 23:03	56.88	5.58	1D-6	2.62	0.673	2/8/08 23:03	56.88	5.64	3D-6	1.89	0.484
2/9/08 16:06	73.93	7.25	1D-7	2.50	0.641	2/9/08 16:06	73.93	7.33	3D-7	0.97	0.250
2/6/08 17:44	3.57	0.23	1E-1	0.00	0.000	2/6/08 17:44	3.57	0.24	3E-1	0.00	0.000
2/7/08 10:35	20.42	1.34	1E-2	4.79	1.228	2/7/08 10:35	20.42	1.35	3E-2	4.33	1.111
2/7/08 14:58	24.80	1.62	1E-3	4.78	1.224	2/7/08 14:58	24.80	1.64	3E-3	4.82	1.236
2/7/08 22:57	32.78	2.14	1E-4	4.46	1.142	2/7/08 22:57	32.78	2.17	3E-4	4.53	1.162
2/8/08 14:15	48.08	3.14	1E-5	4.67	1.198	2/8/08 14:15	48.08	3.18	3E-5	3.48	0.891
2/8/08 23:13	57.05	3.73	1E-6	3.76	0.963	2/8/08 23:13	57.05	3.77	3E-6	3.26	0.836
2/9/08 16:18	74.13	4.85	1E-7	2.79	0.715	2/9/08 16:18	74.13	4.90	3E-7	1.65	0.423
2/6/08 23:34	9.40	0.41	1F-1	0.00	0.000	2/6/08 23:37	9.45	0.42	3F-1	0.12	0.031
2/7/08 10:45	20.58	0.90	1F-2	0.12	0.030	2/7/08 10:47	20.62	0.91	3F-2	0.12	0.030
2/7/08 15:09	24.98	1.09	1F-3	3.60	0.924	2/7/08 15:11	25.02	1.10	3F-3	3.93	1.008
2/7/08 23:08	32.97	1.44	1F-4	4.94	1.268	2/7/08 23:09	32.98	1.45	3F-4	4.56	1.168
2/8/08 14:27	48.28	2.11	1F-5	3.89	0.997	2/8/08 14:29	48.32	2.13	3F-5	2.72	0.698
2/8/08 23:21	57.18	2.49	1F-6	4.27	1.094	2/8/08 23:22	57.20	2.52	3F-6	2.76	0.708
2/9/08 16:30	74.33	3.24	1F-7	3.27	0.838	2/9/08 16:32	74.37	3.28	3F-7	1.81	0.465

Table B.2. Microcystin concentrations analyzed with ELISA for Column Experiment #2.

----- COLUMN #1 -----						----- COLUMN #3 -----					
Date	Time (hrs)	Pore Vol-umes	ID	C (ug/L)	C/Co	Date	Time (hrs)	Pore Vol-umes	ID	C (ug/L)	C/Co
8/12/08 12:42	0.20	--	1P-1	2.13	0.970	8/12/08 12:44	0.23	--	3P-1	2.45	1.114
8/13/08 11:24	22.90	--	1P-2	2.16	0.980	8/13/08 11:28	22.97	--	3P-2	2.09	0.951
8/13/08 21:57	33.45	--	1P-3	2.16	0.981	8/13/08 21:59	33.48	--	3P-3	1.85	0.840
8/14/08 9:43	45.22	--	1P-4	2.16	0.983	8/14/08 9:45	45.25	--	3P-4	1.90	0.864
8/15/08 9:38	69.13	--	1P-5	2.35	1.067	8/15/08 9:40	69.17	--	3P-5	2.27	1.030
8/16/08 10:42	94.20	--	1P-6	2.24	1.017	8/16/08 10:44	94.23	--	3P-6	2.34	1.063
8/17/08 14:56	122.43	--	1P-7	2.24	1.017	8/17/08 14:58	122.47	--	3P-7	2.52	1.146
8/18/08 10:31	142.02	--	1P-8	1.93	0.876	8/18/08 10:33	142.05	--	3P-8	2.02	0.916
8/20/08 11:12	190.70	--	1P-9	1.47	0.669	8/20/08 11:14	190.73	--	3P-9	1.71	0.776
8/22/08 14:53	242.38	--	1P-10	1.11	0.503	8/22/08 14:55	242.42	--	3P-10	1.21	0.549

----- COLUMN #1 -----						----- COLUMN #3 -----					
Date	Time (hrs)	Pore Vol- umes	ID	C (ug/L)	C/Co	Date	Time (hrs)	Pore Vol- umes	ID	C (ug/L)	C/Co
8/12/08 12:58	0.47	0.22	1A-1	0.11	0.052	8/12/08 12:59	0.48	0.23	3A-1	0.11	0.051
8/12/08 13:30	1.00	0.46	1A-2	0.00	0.000	8/12/08 13:30	1.00	0.47	3A-2	0.00	0.000
8/12/08 14:28	1.97	0.90	1A-3	0.17	0.077	8/12/08 14:28	1.97	0.91	3A-3	0.13	0.060
8/12/08 15:03	2.55	1.16	1A-4	1.31	0.595	8/12/08 15:03	2.55	1.17	3A-4	0.66	0.299
8/12/08 15:28	2.97	1.34	1A-5	2.10	0.955	8/12/08 15:28	2.97	1.36	3A-5	1.58	0.718
8/12/08 15:56	3.43	1.55	1A-6	2.20	0.998	8/12/08 15:56	3.43	1.57	3A-6	1.98	0.902
8/12/08 16:31	4.02	1.80	1A-7	2.01	0.915	8/12/08 16:31	4.02	1.82	3A-7	2.56	1.164
8/12/08 17:03	4.55	2.03	1A-8	1.88	0.854	8/12/08 17:03	4.55	2.05	3A-8	2.09	0.952
8/12/08 21:17	8.78	3.76	1A-9	2.08	0.944	8/12/08 21:17	8.78	3.80	3A-9	2.30	1.043
8/13/08 11:43	23.22	9.15	1A-10	1.51	0.687	8/13/08 11:43	23.22	9.25	3A-10	1.07	0.487
8/13/08 21:44	33.23	12.86	1A-11	1.68	0.763	8/13/08 21:44	33.23	13.00	3A-11	0.89	0.405
8/14/08 10:00	45.50	17.68	1A-12	1.65	0.752	8/14/08 10:00	45.50	17.88	3A-12	0.57	0.258
8/15/08 10:02	69.53	27.93	1A-13	1.26	0.574	8/15/08 10:02	69.53	28.23	3A-13	0.48	0.219
8/18/08 13:12	144.70	49.52	1A-14	1.13	0.516	8/18/08 13:12	144.70	50.06	3A-14	1.04	0.474
8/20/08 11:47	191.28	51.60	1A-15	0.78	0.354	8/20/08 11:58	191.47	52.18	3A-15	0.48	0.217
8/22/08 15:20	242.83	58.83	1A-16	0.53	0.243	8/22/08 15:21	242.85	59.47	3A-16	0.00	0.000
8/12/08 13:11	0.68	0.16	1B-1	0.00	0.000	8/12/08 13:12	0.70	0.16	3B-1	0.00	0.000
8/12/08 14:05	1.58	0.36	1B-2	0.00	0.000	8/12/08 14:05	1.58	0.37	3B-2	0.00	0.000
8/12/08 15:38	3.13	0.71	1B-3	0.00	0.000	8/12/08 15:38	3.13	0.72	3B-3	0.00	0.000
8/12/08 16:22	3.87	0.87	1B-4	0.00	0.000	8/12/08 16:22	3.87	0.88	3B-4	0.00	0.000
8/12/08 17:15	4.75	1.06	1B-5	0.00	0.000	8/12/08 17:15	4.75	1.07	3B-5	0.00	0.000
8/12/08 21:07	8.62	1.85	1B-6	2.38	1.083	8/12/08 21:07	8.62	1.87	3B-6	3.03	1.375
8/12/08 22:00	9.50	2.02	1B-7	1.92	0.871	8/12/08 22:00	9.50	2.04	3B-7	3.86	1.753
8/13/08 12:06	23.60	4.65	1B-8	1.70	0.774	8/13/08 12:06	23.60	4.70	3B-8	1.44	0.653
8/13/08 21:32	33.03	6.39	1B-9	2.13	0.969	8/13/08 21:32	33.03	6.46	3B-9	1.20	0.544
8/14/08 10:18	45.80	8.90	1B-10	1.33	0.605	8/14/08 10:18	45.80	9.00	3B-10	0.82	0.375
8/15/08 10:35	70.08	14.08	1B-11	1.21	0.551	8/15/08 10:35	70.08	14.24	3B-11	0.51	0.232
8/16/08 11:05	94.58	19.07	1B-12	1.19	0.539	8/16/08 11:05	94.58	19.28	3B-12	0.53	0.239
8/17/08 15:02	122.53	23.15	1B-13	--	--	8/17/08 15:12	122.70	23.42	3B-13	1.15	0.525
8/18/08 13:24	144.90	24.77	1B-14	1.09	0.494	8/18/08 10:44	142.23	24.91	3B-14	1.17	0.534
8/20/08 12:13	191.72	25.81	1B-15	1.20	0.548	8/20/08 12:25	191.92	26.10	3B-15	0.88	0.398
8/22/08 15:44	243.23	29.44	1B-16	0.60	0.272	8/22/08 15:46	243.27	29.76	3B-16	0.00	0.000
8/12/08 13:46	1.27	0.20	1C-1	0.00	0.000	8/12/08 13:46	1.27	0.20	3C-1	0.00	0.000

----- COLUMN #1 -----						----- COLUMN #3 -----					
Date	Time (hrs)	Pore Vol-umes	ID	C (ug/L)	C/Co	Date	Time (hrs)	Pore Vol-umes	ID	C (ug/L)	C/Co
8/12/08 16:42	4.20	0.63	1C-2	0.00	0.000	8/12/08 16:42	4.20	0.63	3C-2	0.00	0.000
8/12/08 20:59	8.48	1.21	1C-3	1.83	0.832	8/12/08 20:59	8.48	1.23	3C-3	1.14	0.517
8/12/08 21:35	9.08	1.29	1C-4	2.11	0.959	8/12/08 21:35	9.08	1.31	3C-4	1.76	0.800
8/12/08 22:20	9.83	1.39	1C-5	3.05	1.387	8/12/08 22:20	9.83	1.41	3C-5	2.60	1.184
8/13/08 12:29	23.98	3.14	1C-6	1.84	0.835	8/13/08 12:29	23.98	3.18	3C-6	1.23	0.557
8/13/08 21:24	32.90	4.25	1C-7	1.38	0.626	8/13/08 21:24	32.90	4.29	3C-7	1.03	0.470
8/14/08 10:38	46.13	5.98	1C-8	1.27	0.579	8/14/08 10:38	46.13	6.05	3C-8	0.72	0.329
8/15/08 10:55	70.42	9.44	1C-9	1.10	0.501	8/15/08 10:55	70.42	9.54	3C-9	0.48	0.220
8/16/08 11:19	94.82	12.74	1C-10	0.79	0.360	8/16/08 11:19	94.82	12.88	3C-10	0.41	0.188
			1C-11						3C-11		
8/17/08 15:13	122.72	15.45	1C-11	0.71	0.324	8/17/08 15:13	122.72	15.61	11	0.60	0.273
8/18/08 10:52	142.37	16.44	1C-12	0.79	0.358	8/18/08 10:52	142.37	16.61	1C-12	0.92	0.418
			1C-13						3C-13		
8/20/08 12:40	192.17	17.22	13	0.91	0.413	8/20/08 12:40	192.17	17.41	13	1.16	0.526
8/22/08 16:02	243.53	19.64	1C-14	0.32	0.147	8/22/08 16:04	243.57	19.85	3C-14	0.13	0.060
			14						14		
8/12/08 15:20	2.83	0.32	1D-1	0.00	0.000	8/12/08 15:20	2.83	0.33	3D-1	0.11	0.051
8/12/08 21:28	8.97	0.96	1D-2	0.00	0.000	8/12/08 21:28	8.97	0.97	3D-2	0.10	0.047
8/13/08 12:47	24.28	2.39	1D-3	1.91	0.867	8/13/08 12:47	24.28	2.41	3D-3	1.38	0.629
8/13/08 21:17	32.78	3.17	1D-4	1.72	0.781	8/13/08 21:17	32.78	3.21	3D-4	1.30	0.590
8/14/08 12:12	47.70	4.65	1D-5	1.70	0.773	8/14/08 12:12	47.70	4.70	3D-5	0.75	0.343
8/15/08 11:19	70.82	7.12	1D-6	1.33	0.606	8/15/08 11:19	70.82	7.20	3D-6	0.47	0.214
8/16/08 12:02	95.53	9.62	1D-7	1.12	0.508	8/16/08 12:02	95.53	9.73	3D-7	0.41	0.186
8/17/08 15:25	122.92	11.60	1D-8	0.97	0.441	8/17/08 15:25	122.92	11.72	3D-8	0.23	0.103
8/18/08 11:37	143.12	12.34	1D-9	0.65	0.294	8/18/08 11:37	143.12	12.48	3D-9	0.30	0.138
8/20/08 13:09	192.65	12.92	1D-10	0.79	0.361	8/20/08 13:09	192.65	13.06	3D-10	0.59	0.269
8/22/08 16:20	243.83	14.74	1D-11	0.29	0.131	8/22/08 16:20	243.83	14.90	3D-11	0.22	0.101
8/12/08 15:49	3.32	0.25	1E-1	0.00	0.000	8/12/08 15:49	3.32	0.25	3E-1	0.00	0.000
8/12/08 21:43	9.22	0.66	1E-2	0.00	0.000	8/12/08 21:43	9.22	0.66	3E-2	0.00	0.000
8/13/08 10:32	22.03	1.45	1E-3	1.98	0.899	8/13/08 10:32	22.03	1.47	3E-3	1.77	0.804
8/13/08 21:11	32.68	2.11	1E-4	1.62	0.735	8/13/08 21:11	32.68	2.13	3E-4	1.13	0.515
8/14/08 12:34	48.07	3.12	1E-5	1.30	0.592	8/14/08 12:34	48.07	3.16	3E-5	0.76	0.347
8/15/08 11:46	71.27	4.78	1E-6	1.10	0.499	8/15/08 11:46	71.27	4.83	3E-6	0.41	0.186
8/16/08 12:22	95.87	6.44	1E-7	0.92	0.418	8/16/08 12:22	95.87	6.51	3E-7	0.24	0.109
8/17/08 15:35	123.08	7.74	1E-8	0.87	0.397	8/17/08 15:35	123.08	7.82	3E-8	0.22	0.099
8/18/08 12:07	143.62	8.24	1E-9	0.78	0.354	8/18/08 12:07	143.62	8.33	3E-9	0.18	0.080
8/20/08 13:29	192.98	8.62	1E-10	0.39	0.179	8/20/08 13:29	192.98	8.71	3E-10	0.12	0.054
			10						10		

----- COLUMN #1 -----						----- COLUMN #3 -----					
Date	Time (hrs)	Pore Vol- umes	ID	C (ug/L)	C/Co	Date	Time (hrs)	Pore Vol- umes	ID	C (ug/L)	C/Co
8/22/08 16:37	244.12	9.83	1E- 11	0.22	0.099	8/22/08 16:37	244.12	9.94	3E- 11	0.16	0.074
8/12/08 16:49	4.32	0.21	1F-1	0.00	0.000	8/12/08 16:49	4.32	0.22	3F-1	0.00	0.000
8/12/08 21:52	9.37	0.44	1F-2	0.15	0.069	8/12/08 21:52	9.37	0.45	3F-2	0.00	0.000
8/13/08 10:58	22.47	0.99	1F-3	0.26	0.119	8/13/08 10:58	22.47	1.00	3F-3	0.32	0.145
8/13/08 13:06	24.60	1.07	1F-4	1.95	0.885	8/13/08 13:06	24.60	1.08	3F-4	2.07	0.942
8/13/08 16:42	28.20	1.22	1F-5	2.71	1.232	8/13/08 16:42	28.20	1.23	3F-5	2.37	1.078
8/13/08 21:03	32.55	1.40	1F-6	2.09	0.952	8/13/08 21:03	32.55	1.42	3F-6	1.57	0.712
8/14/08 12:59	48.48	2.10	1F-7	1.50	0.683	8/14/08 12:59	48.48	2.12	3F-7	0.99	0.449
8/15/08 12:06	71.60	3.20	1F-8	1.35	0.613	8/15/08 12:06	71.60	3.24	3F-8	0.59	0.268
8/16/08 12:39	96.15	4.30	1F-9	1.16	0.526	8/16/08 12:39	96.15	4.35	3F-9	0.34	0.157
8/17/08 15:44	123.23	5.16	1F- 10	1.04	0.474	8/17/08 15:44	123.23	5.22	3F- 10	0.33	0.149
8/18/08 12:29	143.98	5.50	1F- 11	0.79	0.360	8/18/08 12:29	143.98	5.56	3F- 11	0.26	0.118
8/20/08 13:45	193.25	5.75	1F- 12	0.42	0.189	8/20/08 13:45	193.25	5.81	3F- 12	0.23	0.105
8/22/08 16:52	244.37	6.56	1F- 13	0.36	0.161	8/22/08 16:52	244.37	6.63	3F- 13	0.22	0.101

Appendix C – LC/MS/MS Results, Column Experiments

Table C.1. Algal toxin concentrations (in ug/L) analyzed by liquid chromatography with tandem mass spectrometry.

Sample Location	Collection Date	Collection Time	Anatoxina	Cylindrospermopsin	Deoxycylindrospermopsin	Domoic Acid	Lyngbyatoxin	Microcystin-LA	Microcystin-LF	Microcystin-LR	Microcystin-LW	Microcystin-LY	Microcystin-RR	Microcystin-YR	Nodularin
Bank #2, total (3 freeze-thaw cycles, then filtered)	2/6/2008	1231	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	4.1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Bank #2, dissolved (filtered)	2/6/2008	1231	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	3.8	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #1, Ponded, Sample 1	2/6/2008	1416	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	5.5	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #1, port A, Sample 1	2/9/2008	1523	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.95	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #1, port B, Sample #8	2/9/2008	1540	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.33	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #1, port D, Sample #7	2/9/2008	1606	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.23	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #1, port F, Sample #7	2/9/2008	1630	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.37	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #3, Ponded, Sample 1	2/8/2008	2332	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	5.1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #3, port A, Sample 1	2/9/2008	1523	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	1.0	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #3, port B, Sample #3	2/6/2008	2254	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	5.2	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #3, port B, Sample #5	2/7/2008	2227	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.92	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #3, port B, Sample #8	2/9/2008	1540	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.15	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #3, port D, Sample #3	2/7/2008	1444	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	2.5	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #3, port D, Sample #5	2/8/2008	1402	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.20	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #3, port D, Sample #7	2/9/2008	1606	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.011	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Column #3, port F, Sample #7	2/9/2008	1632	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.028	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01